ABSTRACT

Objective: To design a topical vehicle that provided the optimal balance of betamethasone dipropionate penetration and retention in the skin, with minimal systemic absorption. **Design:** Six test formulations of betamethasone dipropionate 0.05% in vehicles contained the following penetration enhancers: elaidyl alcohol (Formulation-1), hexanol (Formulation-2), dodecanol (Formulation-3), octadecanol (Formulation-4), docosanol (Formulation-5), or oleyl alcohol (Formulation-6). Test agents were applied to human cadaver skin in static Franz-cell chambers containing receptor fluid. **Measurements:** Betamethasone absorption into the receptor fluid was measured over 24 hours. The distribution of betamethasone and its metabolites in the stratum corneum, epidermis, and dermis was analyzed using LC-MS/MS. The formulation with the optimal balance of penetration, permeation, retention, and minimal absorption was selected for a similar study comparing its penetration and absorption versus several commercially available betamethasone formulations. **Results:** Formulation-3 resulted in the highest retention of betamethasone in the skin as well as the highest steroid levels

[Abstract continued on next page]

ORIGINAL RESEARCH

Rational Vehicle Design Ensures Targeted Cutaneous Steroid Delivery

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TOPICAL CORTICOSTEROIDS ARE the mainstay of treatment for multiple cutaneous immune diseases, such as psoriasis.[1–3] Topical corticosteroids have been used since the 1960s.[4,5] Back then, the debate over efficacy surrounded the percentage of steroid used, while recommendations regarding the overall formulation were to dilute the preferred steroid in "an appropriate base."[5] Little description was given to the vehicles used to deliver steroid to the skin. Over time, new formulations and types of corticosteroids were developed, leading to improved and more varied ointments, creams, and lotions with superior efficacy and cosmetic acceptability.[6–8] Today, topical drug delivery is a developing field where the improved science of drug formulation combined with advances in our understanding of the skin barrier have led to optimization of drug delivery by coupling unique vehicle properties with the biology of the active molecule. It is now becoming increasingly possible to tailor the ingredients of the vehicle formulation to facilitate drug delivery to the site of the disease. Topical vehicles that optimize corticosteroid delivery to the affected pathology while reducing systemic absorption could enhance the treatment of many cutaneous immune

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[Abstract continued]

in the receptor fluid at 12 and 24 hours. Formulation-6 had the second highest retention of betamethasone in total skin, with relatively low absorption into the receptor fluid. All other variants had both lower steroid retention in the skin and lower absorption into the receptor fluid, with the exception of Formulation-2 which had higher absorption at 24 hours. Formulation-6/DFD-01 was selected for further development. Comparison of Formulation-6/DFD-01 with commercially available formulations of betamethasone dipropionate showed it had the highest steroid levels in the epidermis and dermis combined, with relatively low levels in the receptor fluid. **Conclusion:** Formulation-6/DFD-01 had the optimal balance of betamethasone retention in the skin, with low systemic absorption. This designed vehicle ensured retention of the corticosteroid in skin layers to maximize local efficacy while minimizing potential for hypothalamic-pituitary-adrenal suppression.

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diseases, such as psoriasis.

The foundation of management of inflammatory dermatitis is to deliver anti-inflammatory molecules in the form of corticosteroids to the site of the pathology within the skin. The physiological consequences of applying any active drug onto the surface of the skin involves three essential biologic activities: *penetration* of the stratum corneum, *permeation* into the epidermis and dermis, and subsequent *absorption* as a certain percentage of the drug passes into the systemic circulation.

The most important side effects associated with topical corticosteroid use are based around this systemic absorption, which can then cause suppression of the hypothalamic–pituitary–adrenal (HPA) axis. Topical corticosteroids typically carry a warning that systemic steroid absorption can, in some patients, result in Cushing's syndrome, hyperglycemia, or unmasking of latent diabetes mellitus. The risk is higher for higher potency corticosteroids, and for patients who apply steroids to large areas for long periods of time. While these systemic adverse events are reversible, usually alleviated once treatment is discontinued, reducing the amount of corticosteroid absorbed will reduce the risk of these systemic adverse events.

Potential local adverse events associated with topical corticosteroids include atrophy, striae, telangiectasia, burning, itching, irritation, folliculitis, acneiform eruptions, hypopigmentation, and allergic contact dermatitis. These are also frequently associated with prolonged use of topical corticosteroids, as well as use on more fragile skin locales, such as the face, genitals, or intertriginous areas. The majority of these conditions are reversible when treatment is discontinued, although striae, atrophy, and telangiectasia are likely to be permanent.

An ideal topical agent would penetrate the stratum corneum without damaging its skin barrier function. Once through the stratum corneum, this ideal corticosteroid would permeate the underlying epidermis and dermis and be retained there, with minimal absorption into the systemic circulation.

The intact stratum corneum has a high diffusional resistance[9] created by alternating lipoidal and hydrophilic regions; a special formulation is required to breach it successfully without compromising its structure. To ensure that the drug penetrates the stratum corneum in sufficient quantities to effect an immune response in the underlying skin layers, penetration enhancers are added to the steroid formulation. The type and concentration of penetration enhancer significantly influences the skin penetration/permeation and systemic absorption of drug.

The aim of this study was to evaluate the impact of formulation composition on the penetration, permeation, retention, and absorption of betamethasone dipropionate through cadaver skin over time. Six formulations (Formulations-1 to -6) were tested, each of which contained a different penetration enhancer, to find which of the six provided the optimal balance of maximized penetration, permeation, and retention, but with minimal absorption. The optimally balanced formulation of betamethasone dipropionate 0.05% (Formulation-6/DFD-01) was then compared with commercially available topical corticosteroids.

METHODS

Formulation *in vitro* evaluation and selection. The test agents all contained betamethasone dipropionate 0.05% in the base vehicle of water, sorbitan monosterate, polyoxyl 20 cetostearyl ether, cetostearyl alcohol, mineral oil, propyl paraben, methyl paraben, butylated hydroxyl toluene, and hydroxyethyl cellulose. Permeation enhancers were added to this base to create six test steroid formulations: elaidyl alcohol (Formulation-1), hexanol (Formulation-2), dodecanol (Formulation-3), octadecanol (Formulation-4), docosanol (Formulation-5), or oleyl alcohol (Formulation-6).

The six test formulations (2.5mg/cell or 5mg/cm^2) were applied to human cadaver skin in static Franz-cell chambers (8mm diameter) containing 3.0mL receptor fluid (4% bovine serum albumin in water with 0.01% gentamicin sulfate), continuously mixed. The temperature was set at 32±0.1ºC, and tissue samples were equilibrated for one hour before dosing. At the end of this hour, samples of receptor fluid were taken as the t=0 samples, and fresh receptor fluid added to the chambers.

The receptor fluid simulates the absorption of betamethasone through the skin and passing into the systemic circulation *in vivo*. The amount of betamethasone in the receptor fluid was measured at 0, 2, 6, 10, 12, and 24 hours by removing all the receptor fluid, and replacing it with a fresh batch of receptor fluid at 32ºC.

At 24 hours, the distribution of betamethasone dipropionate and its metabolites (betamethasone, betamethasone-17-propionate, betamethasone-21-propionate, and betamethasone-17,21-dipropionate) in the stratum corneum, the epidermis below the stratum corneum, and the dermis was analyzed using liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). The skin tissue was removed from the HT Franz cell, dried and the layers separated. Standard tape stripping was used to remove the stratum corneum; a surgical blade was then used to scrape the epidermis from the dermis before the remaining dermis layer was cut into small pieces. Each layer was individually extracted with 4.0mL of DMSO/acetonitrile (50/50 v/v) at room temperature overnight with agitation from an orbit shaker. Supernatants were collected for analysis. All testing was repeated nine times. The quantity of betamethasone dipropionate and its metabolites in each skin layer were added together and described as "total betamethasones" or "betamethasones."

Analytical LC-MS method. LC-MS was performed utilizing a AB Sciex SPI3200™ instrument (ABI Sciex, Framingham, Massachusetts) coupled to a Shimadzu HPLC system (Columbia, Maryland) fitted with a Venusil XBP 3µm, 100Å, 4.6×150mm C18(2) column (Bonna-Agela Technologies Inc., Wilmington, Delaware). Samples were eluted with a 6.5-minute gradient of acetonitrile and 0.1% acetic acid 1mM NH_4OAc in water at a flow rate of 0.75mL/min, then the ions were generated by electron spray ionization technique and scanned at negative polarity in multiple reaction monitoring (MRM) mode. Tissue samples were prepared as described below followed by a reversed phase gradient liquid–liquid extraction technique to extract the following analytes from the samples betamethasone, betamethasone-17-propionate, betamethasone-21-propionate, and betamethasone-17,21-dipropionate.

Comparison of Formulation-6 with commercially available betamethasone dipropionate products. The test agent combining optimum levels of penetration and retention from the experiment above (Formulation-6) was compared with commercially available betamethasone products in a similar test apparatus to that described above. Formulation-6 (betamethasone dipropionate 0.05%) was an oil-in-water emulsion formulation. The comparator products were diprolene (augmented betamethasone dipropionate 0.05% [AugBD; Merck, Whitehouse Station, New Jersey]) lotion (containing hydroxypropyl cellulose, isopropyl alcohol [30%], phosphoric acid, propylene glycol, purified water, and sodium phosphate monobasic monohydrate); AugBD ointment (containing propylene glycol, propylene glycol stearate, white petrolatum, and white wax), and AugBD cream (containing carbomer 940, ceteareth-30, chlorocresol, cyclomethicone, glycerol

Table 1. Detail of total betamethasones distributed in skin layers and receptor fluid after 24 hours

FORMULATION, NG	RECEPTOR FLUID	SKIN SURFACE	STRATUM CORNEUM (SC)	EPIDERMIS (WITHOUT THE STRATUM CORNEUM) (E)	DERMIS (WITHOUT THE EPIDERMIS) (D)	TOTAL PENETRATION IN SKIN (SC + E + D)
Formulation-1	11.26	1436.97	5.4	17.09	14.22	36.71
Formulation-2	19.35	1459.7	11.11	15.23	4.87	31.21
Formulation-3	50.69	1133.61	36.83	42.55	33.81	113.19
Formulation-4	2.31	588.41	2.02	1.89	0.79	4.7
Formulation-5	2.78	1300.04	17.57	7.43	6.38	31.39
Formulation-6	11.82	1212.44	33	18.34	14.11	65.45

oleate/propylene glycol, purified water, sodium hydroxide, sorbitol solution, white petrolatum, and white wax).

Absorption of betamethasone into the receptor fluid was measured at 0, 2, 6, 10, 12, 22, and 24 hours, and distribution of betamethasone and its metabolites in the stratum corneum, epidermis, and dermis were analyzed using LC-MS/MS as described above. All testing was performed on 12 replicate skin samples.

RESULTS

Formulation selection.

Distribution of betamethasones in the skin and receptor fluid after 24 hours is detailed in Table 1 and Figures 1 and 2. Testing of permeation enhancers revealed the highest retention of betamethasones in total skin occurred with Formulation-3 (Figure 1), followed by Formulation-6. Formulation-4 had the lowest retention of betamethasones in the skin.

When absorption was studied (total betamethasones that penetrated the skin, permeated through the skin layers, and accumulated in the receptor fluid), the highest betamethasone absorption was with Formulation-3 at 12 and 24 hours (Figure 2), followed by Formulation-2 at 24 hours. Formulation-1 and Formulation-6 demonstrated similar low levels of absorption, and Formulation-5 showed the lowest receptor fluid levels of total betamethasones.

Study of the individual skin layers for product revealed the skin layer concentrations of betamethasones for Formulation-3 were 37, 43, and 34ng in the stratum corneum, epidermis, and dermis,

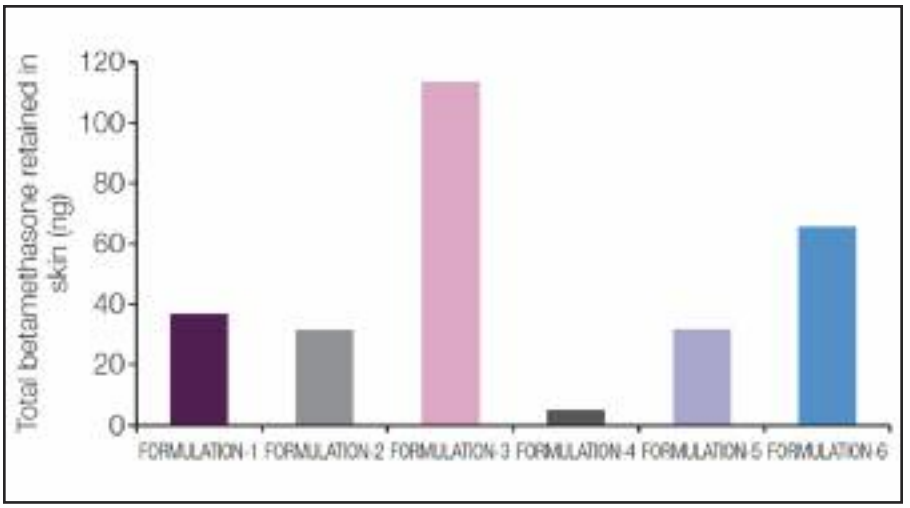

Figure 1. Penetration of total betamethasones from test formulations into the skin over 24 hours

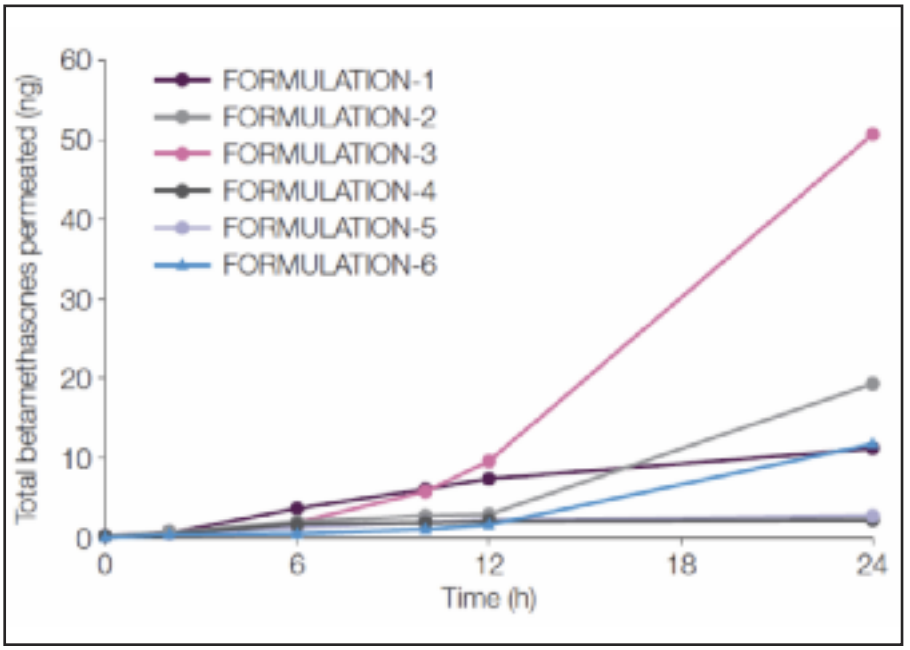

Figure 2. Absorption of total betamethasones into receptor fluid over 24 hours for each test formulation

respectively (Table 1). For Formulation-6, the concentrations were 33ng in the stratum corneum, 18ng in the epidermis, and 14ng in the dermis. The skin layer concentrations of betamethasones in the epidermis and dermis showed similar patterns to the overall skin retention results. High amounts of formulation remained on the skin surface (Table 1).

Formulation-6 demonstrated the optimal balance between overall penetration and retention in the skin, with low absorption through the skin into the receptor fluid, and was thus selected for commercial development and renamed DFD-01 (Sernivo™, Promius Pharma, LLC, Princeton, New Jersey).

Formulation comparison. When compared with commercially available betamethasone cream, lotion, and ointment, Formulation-6/DFD-01 resulted in relatively high levels of total betamethasones in the skin with similar levels accumulating in the epidermis and dermis (Figure 3).

AugBD ointment had lower levels of betamethasones in the epidermis compared with Formulation-6/DFD-01. AugBD lotion and AugBD cream both had low levels of betamethasones in the epidermis and dermis compared with Formulation-6/DFD-01.

With Formulation-6/DFD-01, 60ng of total betamethasones remained in the skin layers and 30ng passed into the receptor fluid (Figure 4). AugBD ointment was closest in skin profile to Formulation-6/DFD-01, with almost 60ng retained in the skin, and <40ng total betamethasones in the receptor fluid. The results for AugBD cream showed a low level of absorption, with only 30mg retained in the skin, coupled with <10ng betamethasones in the receptor fluid; AugBD lotion mainly passed into the receptor fluid (>65ng), with only 40ng retained in the skin layers.

DISCUSSION

Actively targeting drugs to specific layers of the skin is an emerging and challenging field,[10] which has the potential to improve patient care in the future. The results here show that Formulation-6/DFD-01 has less active drug reaching the apparatus reservoir fluid than some commercially available

formulations. Formulation-6/DFD-01, containing oleyl alcohol, sorbitan monosterate, polyoxyl 20 cetostearyl ether, cetostearyl alcohol, and mineral oil, demonstrated a good balance of betamethasone(s) retention in the epidermis and dermis to maximize local efficacy with low systemic absorption. The carbon chain length and degree of unsaturation of the penetration enhancer oleyl alcohol influences the permeation of steroid, resulting in the retention of corticosteroid in the desired skin layers while minimizing passage into the receptor fluid, which acts as a surrogate for systemic absorption.[11–19] Minimizing systemic absorption reduces the potential for systemic adverse events, including HPA axis suppression.

Compared with commercially available topical corticosteroid formulations, Formulation-6/DFD-01 performed well, with more product retained in the skin than AugBD cream or lotion, and less corticosteroid passing through to the reservoir, in contrast to the results for AugBD lotion. The low level of betamethasones in the receptor fluid with Formulation-6/DFD-01 may have important safety implications for this drug.

The exact site of action of psoriasis and other cutaneous immune diseases within the skin is controversial, and research has not fully characterized the pathology of these diseases within each skin layer. The location of some immune actions thought to be important in psoriasis have been localized to the epidermis and dermis of the skin[20–23] and therefore any effective treatment would need to provide steroid to both these skin layers. In a clinical trial comparing Formulation-6/DFD-01 versus AugBD lotion for the treatment of moderate psoriasis, both products demonstrated similar efficacy after 14 days of BID treatment.[24] This result further challenges the tenet that the higher the potency of the steroid (as determined by vasoconstriction assay [VCA] skin blanching score), the greater its clinical efficacy, as VCA analyses indicated that Formulation-6/DFD-01 is a mid-potent corticosteroid compared with AugBD lotion, which has super-high potency.[25] The vehicle composition

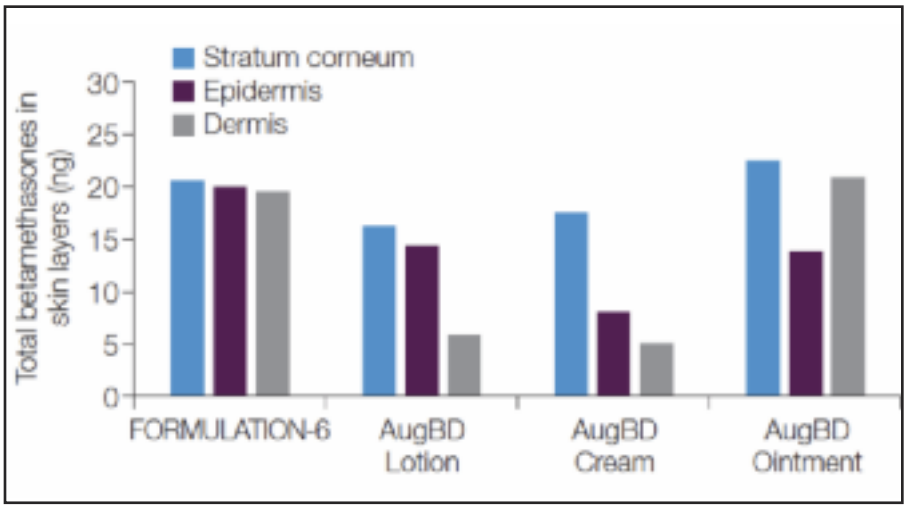

Figure 3. Formulation-6/DFD-01 had the highest permeation of total betamethasones into the epidermis after 24 hours versus other commercial formulations

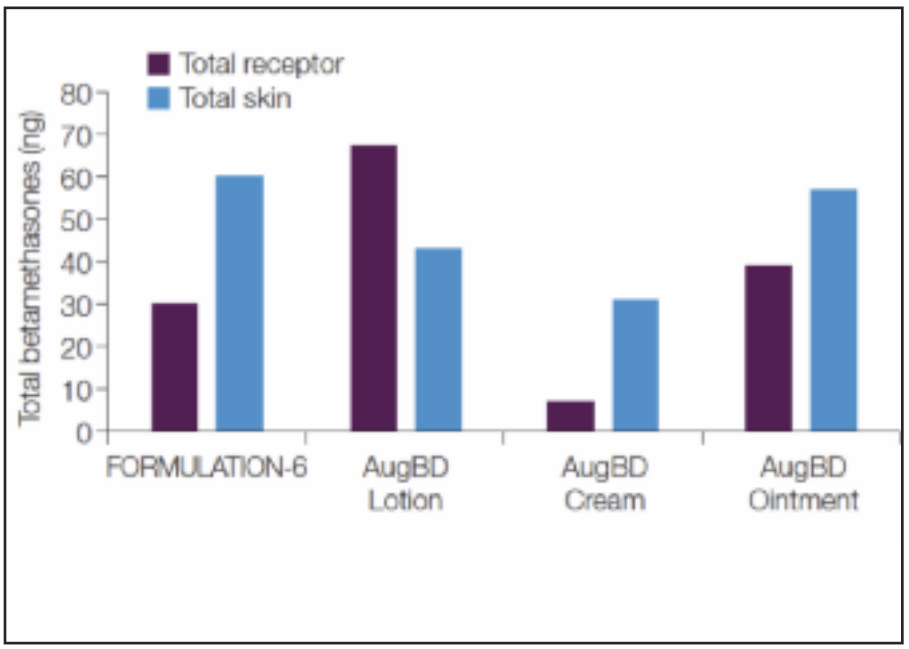

Figure 4. Formulation-6/DFD-01 total betamethasones (ng) levels in receptor fluid and all layers of skin after 24 hours versus other commercial formulations

of Formulation-6/DFD-01 appears to result in the preferential delivery of more active drug to the epidermis and dermis, the purported site of psoriatic disease activity. This targeted delivery may be one explanation for Formulation-6/DFD-01 demonstrating clinical efficacy similar to a high potency steroid, but with the VCA response of a mid-potent steroid.

A high retention of corticosteroid in the skin may give rise to concerns of atrophy. Clinical trials with Formulation-6/DFD-01 show the rates of atrophy were very low (~1%) and do not indicate increased atrophy with this formulation.[24,26] Formulation-6/DFD-01 does not cause a reservoir of corticosteroid in the skin, but reduces the amount of corticosteroid being absorbed into the systemic system.

It should be noted that a high proportion of corticosteroid from all the test products remained on the skin surface. Indeed, most topical steroids maintain a reservoir on the skin surface, with only a fraction of active drug (<5%) penetrating the skin layers.[27]

There were limitations to this study. The use of cadaver skin, which is not fully representative of diseased skin in patients, is nevertheless a currently established methodology for investigating how drug moves through the skin over time. Cadaver skin lacks a living blood supply and integration with body systems; however, multiple studies have demonstrated comparable permeation and absorption patterns of various compounds between living and cadaver skin.[18,19,28,29] Coupled with LC-MS/MS analysis, it provides an accurate method to track the breakdown and metabolism of corticosteroid within the skin.

CONCLUSION

Formulation-6/DFD-01 optimized cutaneous penetration, permeation, and retention of betamethasone with low systemic absorption. It delivered higher levels of betamethasones to the dermal layer compared with several other commercially available betamethasone dipropionate products. This mid-potent corticosteroid with its delivery optimized technology-based formulation produces clinical efficacy results similar to those of a higher-potency corticosteroid.[24]

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